Synopsis

Structure Based Virtual Screening and Validation of Potential Quorum Sensing Inhibitors Against LasR in *Pseudomonas aeruginosa*

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Introduction

Biofilm is an aggregation of bacteria within a self-secreted extracellular polymeric substances (EPS) matrix which serve as a strategy for survival of the species in adverse conditions (Moradali *et al.,* 2020). The community of bacteria in biofilms can be of same or different species and utilize any adherent surface as nutrient source to grow (Banerjee *et al.,* 2020). Bacteria in biofilms are more resistant to antimicrobials compared to their planktonic state and interrupts the host immune responses (Thi *et al.,* 2020). As a result, a wide range of diseases associated with biofilms namely bloodstream infections, urinary tract infections and native host tissue infections are increasing till date (Sharma *et al.,* 2019).

National Institute of Health (NIH) has stated bacterial biofilms cause approximately 65% of microbial diseases and contribute to more than 80% of chronic infections (Jamal *et al.,* 2018). According to the World Health Organization report (2017), *Pseudomonas aeruginosa* is a priority and multidrug resistant bacteria for which new antibiotics must be developed immediately. Furthermore, *P. aeruginosa* has been listed as one of the commonly occurring hospital "superbugs" linked to nosocomial infections (Streeter *et al.,* 2016).

The annual incidences of infections caused by multidrug resistant (MDR) pathogenic bacteria would have been increased to ten million deaths by 2050 (Gupta and Singhal, 2018; Spaulding *et al.,* 2018). In addition, the UK government's review report stated that antimicrobial resistance (AMR) deaths will outnumber cancer deaths by 2050. (Ahmed *et al.,* 2019). In India, the prevalence rate of *P.aeruginosa* infection varies from 10.5% to 30% (Senthamarai *et al.,* 2014). On the basis of a study conducted at a tertiary care hospital of India that accounted for 16,210 isolates from different clinical specimens of trauma patients, it was found that *P. aeruginosa* was the most common species associated with various infections of hospital settings (Kumari *et al.,* 2019).

P. aeruginosa is a clinically virulent Gram negative bacterium involved in the pathogenesis of various acute and chronic infections caused by biofilm formation. *P. aeruginosa* biofilms are significantly more prevalent in patients with chronic wounds, lungs of cystic fibrosis and individuals with weakened immune systems. It is one of the most common nosocomial pathogen affecting hospitalized patients and it is a major contributor to increase morbidity and mortality rates. *P. aeruginosa* infections are extremely difficult to treat due to their natural resistance to majority of antibiotics and increasing number of multidrug resistant strains worldwide. They employ sophisticated molecular mechanisms and genotypic events required for survival during antimicrobial therapy and pathogenesis, contributing to the rising incidences of biofilm-associated infections (Moradali *et al.,* 2017).

P. aeruginosa communicates between cells and coordinates collective behaviours in response to changes in environmental conditions and cell density via an interconnected network of pathways known as quorum sensing (QS) (Mukherjee and Bassler, 2019). The QS mechanism involves signalling molecules known as autoinducers (AIs), which are sensed by specific transcriptional regulators and regulates specific set of genes necessary for biofilm formation. Furthermore, QS regulates a variety of physiological processes, including host-microbe interaction, stress tolerance, virulence factor production, and metabolic adjustment (Moradali *et al.,* 2017).

The QS system of *P. aeruginosa* is an interconnected network involving *las*, *rhl*, Pseudomonas quinolone signal (*pqs*), and integrated QS (*iqs*), respectively (Lee and Zhang, 2015). The *las*, *rhl*, and *pqs* network systems are important in biofilm development as they produce a variety of functional elements such as pyocyanin, rhamnolipids, Pel polysaccharides, pyoverdine, and lectins. These networks are well connected in a hierarchical fashion by holding the *las* system at the top position. LasI and LasR forms the *las* system, where its autoinducer molecule N-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL) binds to LasR and activates the transcriptional regulation of multiple genes (Thi *et al.,* 2020).

LasR is the primary protein involved in the regulation of virulence phenotypes such as protease production, biofilm formation, and the production of pyocyanin and rhamnolipids (Asfahl and Schuster, 2018). LasR comprises of two domains namely, an amino-terminal ligand binding domain (LBD) and a C-terminal DNA binding domain (DBD). When the autoinducer OdDHL binds to LasR, it stabilises and dimerizes into two LasR subunits. The resultant ligand-bound homodimer then has the ability to bind DNA and activates transcription to regulate quorum sensing regulatory genes for coordinating group behaviours such as biofilm development, antibiotic tolerance, host-pathogen interactions, metabolic changes, cytotoxicity, nutrient acquisition, and microbial competition (Papenfort and Bassler, 2016). Thus, targeting LasR may be of increased interest because of its position in the hierarchical network and its predominance in QS-associated virulence of *P. aeruginosa.*

Various approaches have been tried over the years to find a potent lead molecule with antiquorum sensing and antibiofilm activities against *P. aeruginosa* infections. Nonetheless, experimental and modelling studies have revealed that the spread of resistance in bacteria to the identified quorum sensing inhibitors (QSIs) is still on (Rasamiravaka *et al.,* 2015). *In silico* computational methods provide a variety of tools for identifying novel drug candidates from which chemical and biological information about ligands and targets can be derived (Lokhande *et al.,* 2019). Using the crystal structure of the QS receptor, structure-based virtual screening (SBVS) can be used to discover novel QSIs. A few groups have already investigated the *in silico* discovery of QSIs against the LasR receptor (Sadiq *et al.,* 2020; Nain *et al.,* 2020; Kalia *et al.,* 2017, Tan *et al.,* 2013).

The current study was designed to identify effective QSIs targeting LasR for the treatment of *P. aeruginosa* infections. Initially, potential inhibitors of LasR were identified using virtual screening (VS), and their stability towards LasR were evaluated by molecular dynamics simulation. *In vitro* assays were performed to validate the selected LasR inhibitors for its antibiofilm and antiquorum sensing efficacy. Furthermore, the antagonistic efficiency of the selected inhibitors to inhibit LasR at the transcriptional level was evaluated by a reporter gene assay and qRT-PCR was done to quantitatively measure the relative gene expression of QS regulatory genes. Finally, the selected LasR inhibitors were assessed for their potential to inhibit QS controlled virulence factors production and their cytotoxicity were determined.

The objectives framed for the current study are as follows:

- To screen for inhibitors against LasR through molecular docking based virtual screening.
- To assess the antibiofilm and antiquorum sensing activity of the selected LasR inhibitors against *P. aeruginosa.*
- To investigate the gene expression profile of quorum sensing regulatory genes in *P. aeruginosa*.
- To examine the effect of selected LasR inhibitors on quorum sensing mediated virulence factors of *P. aeruginosa*.

The study was divided into four phases to meet the above objectives.

Phase I - Virtual screening for inhibitors against LasR of *Pseudomonas aeruginosa* and molecular dynamics simulation study

Molecular docking based virtual screening method was adopted in the phase I to look for a new class of LasR inhibitors that could act as potent antagonists against *P. aeruginosa*. Initially, the crystal structure of the LasR protein (PDB ID: 3IX4) was retrieved from Protein Data Bank (PDB). The protein was then prepared using the protein preparation wizard module of the Schrödinger software. Following that, high-throughput virtual screening (HTVS) was performed in three stages using Schrödinger small molecule database namely structure-based virtual screening, standard precision (SP) docking, and extra precision (XP) docking.

Virtual screening was carried out with 3,034,496 compounds from the Schrödinger small molecule database to identify compounds that showed interactions with LasR. Among them, 1,942,018 compounds are docked in the HTVS mode; the top 10% of the compounds from HTVS screening was subjected to standard precision (SP) docking, which ranked molecules based on docking scores. A total of 19,420 compounds were docked, and the top 10% of the SP docked compounds were further taken for flexible extra

precision (XP) docking. Finally, 1942 compounds were filtered from the XP docking mode. By the end of the process, the top 12 hit compounds with docking score less than -11.0 kcal/mol were selected. From this, top three compounds namely CACPD2011a-0001928786 (3-[2-(3,4-dimethoxyphenyl)-2-(1H-indol-3-yl)ethyl]-1-(2-fluorophenyl)urea), CACPD2011a-0001927437 (3-(4-fluorophenyl)-2-[(3-methylquinoxalin-2-yl)methylsulfanyl]quinazolin-4-one), and CACPD2011a-0000896051 (2-({4-[4-(2 methoxyphenyl)piperazin-1-yl]pyrimidin-2-yl}sulfanyl)-N-(2,4,6-trimethylphenyl)acetamide) were chosen for analysis and designated as C1, C2, and C3, respectively.

The protein-ligand interaction patterns of the selected compounds with LasR were then evaluated and compared to that of the native ligand (OdDHL) and the co-crystallized ligand TP-1 (Triphenyl mimic of autoinducer). According to the ligand interaction profile, OdDHL and TP-1 showed a similar pattern of hydrogen bonding involving four residues: Tyr56, Asp73, Ser129, and Trp60. In the case of selected compounds, C1 and C2 shared only three out of four residues to form hydrogen bonds with LasR, whereas C3 did not exhibit any hydrogen bond contact.

The binding free energies of the selected compounds in complex with LasR were calculated using the Prime Molecular Mechanics/Generalized Born and Surface Area (MM-GBSA) method in Schrödinger software. The binding free energy (ΔG_{Bind}) of the selected compounds dissociated into various energies namely Coulomb energy, lipophilic energy, van der Waals energy, solvation energy, and strain energy of the ligands ranged between -112.2 to -92.2 kcal/mol. C2 had the highest binding free energy (ΔG_{Bind}) among the three compounds, while C3 had the lowest. As a result, based on the various binding energies computed, it was inferred that all three selected compounds had significant binding affinity towards the target protein LasR.

The ADMET and drug-likeness properties of the selected compounds were analysed using QikProp module of Schrödinger software. The results showed that the selected compounds had good pharmacokinetic profiles, with all parameter values falling within the acceptable range. Further, to confirm the stability of the selected compounds in the active site of LasR, molecular dynamics simulation was carried out for 100 ns using Desmond module of Schrödinger software. Root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (ROG), and intramolecular hydrogen bonds were used to assess the overall stability. After 100 ns of simulation, the selected compounds were found to have a stable contact with LasR, mediated by favourable interactions with key amino acid residues, which indicated that they can act as probable inhibitors of LasR. Finally, the selected compounds were purchased from Mcule, Hungary and used for subsequent studies.

Phase II - Assessment of antibiofilm and antiquorum sensing activity of selected LasR inhibitors against *Pseudomonas aeruginosa*

The antibiofilm activity of the selected LasR inhibitors were evaluated using various assays, including biofilm inhibition assay, eradication of preformed matured biofilm, biofilm detachment assay, exopolysaccharide (EPS) quantification assay, alginate assay, estimation of biofilm protein concentration, and evaluation of metabolic activity of viable cells. Imaging with a scanning electron microscope (SEM) revealed the morphology of biofilm in the presence and absence of selected LasR inhibitors. All of the assays were performed in accordance with the standard protocols described in the literature.

Initially, the minimum inhibitory concentration (MIC) of the selected LasR inhibitors were determined using the microbroth dilution method. Two fold serial dilution of the test compounds ranging from 4000 - 1.95 μM were performed in Luria Bertani (LB) broth followed by suspension of inoculum and incubated at 37°C for 16-18 hours. The MIC was defined as the lowest concentration of compound that completely inhibited visible growth of the organism in LB broth. C1 and C2 completely inhibited growth at a concentration of 1000 μM whereas C3 inhibited at 500 μM. Thus, these concentrations were considered as the MICs of the selected compounds. A concentration range below the MIC values namely ½ and ¼ sub-MICs of the compounds were used for further study.

The biofilm inhibition properties of the selected LasR inhibitors were investigated using crystal violet staining assay. The percentage of biofilm inhibition by the selected compounds were determined by measuring their absorbance at 570 nm. The results revealed that the selected compounds namely C1, C2, and C3 inhibited biofilm formation at their ½ sub-MIC up to 84.86%, 83.66%, and 83.06%, respectively and at ¼ sub-MIC up to 80.94%, 80.70%, and 81.81%, respectively.

The eradication potential of the selected LasR inhibitors on preformed matured biofilm of *P. aeruginosa PAO1* were observed by measuring their optical density at 570 nm. Here, the residual planktonic cells obtained from the matured biofilm formed after 24 hours of incubation were discarded, and the adhered biofilm cells were treated with ½ and ¼ sub-MICs of the selected LasR inhibitors. The results revealed a dose-dependent disruption of matured biofilm with inhibition percentage of 67.32%, 48.73%, and 61.05% at ½ sub-MICs of C1, C2, and C3, respectively whereas at ¼ sub-MICs it showed an inhibition percentage of 54.08%, 46.37%, and 51.76% for C1, C2, and C3, respectively. A detachment assay was carried out to determine whether the selected LasR inhibitors had any effect on biofilm adhesion to the substratum. The inhibition of adhesion ability by the selected LasR inhibitors namely C1, C2, and C3 were assessed by measuring their absorbance at 570 nm. The results revealed that the selected compounds inhibited the adhesion ability of biofilm cells at ½ sub-MICs by 84.74%, 84.25%, and 83.94% and at ¼ sub-MICs to 83.40%, 81.62%, and 78.55% for C1, C2, and C3, respectively.

The effect of the selected LasR inhibitors on extracellular polysaccharide (EPS) production by *P. aeruginosa PAO1* was determined. The samples treated with ½ and ¼ sub-MICs of selected LasR inhibitors for their EPS production was quantified using phenol-sulphuric acid method by measuring their absorbance at 490 nm. The results inferred a decrease in the production of EPS when treated with selected compounds. The percentage of inhibition of EPS production by ½ sub-MICs were found to be 69.93%, 67.54%, and 68.24% and at ¼ sub-MICs it was 59.15%, 52.41%, and 59.76%, respectively for C1, C2, and C3.

Alginate is a component of the extracellular matrix in *P. aeruginosa* biofilms, thus the effectiveness of the selected LasR inhibitors in reducing alginate production was evaluated. The decrease in alginate production by the selected compounds were determined by measuring their absorbance at 530 nm. The results revealed that the selected LasR inhibitors namely C1, C2, and C3 reduced alginate production at ½ sub-MICs up to 63.57%, 60.14%, and 54.71% and ¼ sub-MICs up to 46.37%, 45.86%, and 46.41%, respectively.

The amount of protein present in the biofilm were measured according to the method described by Lowry *et al.* (1951). When compared to untreated control (261.73 µg/ml), samples treated with ½ sub-MICs of C1, C2, and C3 have low protein concentrations of 99.65 µg/ml, 112.43 µg/ml, and 112.04 µg/ml, respectively whereas on treatment with ¼ sub-MICs of the selected compounds the concentrations of protein was found to be 109.43 µg/ml, 129.77 µg/ml, and 134.99 µg/ml for C1, C2, and C3, respectively. The metabolic activities of viable biofilm cells was measured using the MTT assay method. Upon treatment with ½ sub-MICs of C1, C2, and C3, the metabolically active cells were found to be only 40.2%, 45.24%, and 45%, respectively whereas ¼ sub-MICs of C1, C2, and C3 exhibited a percentage of cell viability of 48.13%, 50.26%, and 48.38%, respectively. These finding suggested that the selected LasR inhibitors were able to reduce the number of active biofilm cells and resulted in cell inactivation.

Scanning Electron Microscope (SEM) was used to determine the impact of the selected LasR inhibitors on biofilm architecture and cell morphology. The SEM images captured at the imaging conditions of 15.00 kV acceleration potential and 15,000 X magnification revealed that ½ and ¼ sub-MICs of the selected compounds inhibited cell attachment to the surface. Thus, all of the above findings reported that the selected LasR inhibitors possess potential antibiofilm activity against *P. aeruginosa PAO1*.

The antiquorum sensing ability of the selected LasR inhibitors were determined by performing *in vitro* assays such as inhibition of violacein pigment by disc diffusion assay, swimming motility assay, and swarming motility assay in *Pseudomonas aeruginosa PAO1*. *Chromobacterium violaceum* was used as a biomarker strain for preliminary screening of the selected compounds for its antiquorum sensing activity. *C. violaceum* produce a purple pigment referred to as violacein due to its quorum sensing ability. Inhibition of violacein pigment production is observed when their active quorum sensing signals are inhibited. Here the antiquorum sensing potency of the selected compounds were observed by measuring the diameter of zone of

inhibition (cm) of violacein pigment. The results revealed that all three selected LasR inhibitors demonstrated potent quorum sensing inhibitory activity, with zone of inhibition of violacein pigment as 1.46 ± 0.04 cm, 1.5 ± 0.08 cm, and 1.03 ± 0.04 cm at ½ sub-MICs of C1, C2, and C3, respectively compared to vehicle control (DMSO) which showed no effect on violacein pigment production.

P. aeruginosa flagellar motility is required for biofilm formation and development. In this context, the swimming and swarming motilities were investigated in order to determine the effect of the selected LasR inhibitors on the motility behaviour of *P. aeruginosa*. The diameter of swimming motility (cm) was found to be 4.13 ± 0.57 cm in case of untreated control. Whereas, in presence of the selected compounds namely C1, C2, and C3 at ½ sub-MICs, the diameter of swimming motility were decreased to 3.1 ± 1.0 cm, 3.36 ± 0.57 cm, and 3.36 ± 0.52 cm, respectively. The samples treated with ¼ sub-MICs of the selected compounds C1, C2, and C3 also decreased swimming motility to 3.48 ± 0.76 cm, 3.76 ± 0.57 cm, and 3.56 ± 0.57 cm, respectively.

Quorum sensing regulated swarming motility is a type of flagella-dependent movement in viscous environments. This type of motility promotes bacterial adhesion to various surfaces or host cells, which is the first step in the formation of a biofilm. The results revealed that ½ sub-MIC of C1 inhibited swarming motility to 0.48 ± 0.28 cm compared to that of the untreated control (3.76 ± 0.57 cm) whereas sample treated with ¼ sub-MIC of C1 showed swarming motility of 0.66 ± 0.57 cm. Further, at ½ sub-MIC of C2, the swarming motility was reduced to 0.56 ± 0.57 cm and at ¼ sub-MIC, the swarming motility was found to be 0.66 ± 1.85 cm. The compound C3 at ½ and ¼ sub-MICs had a swarming motility of 0.65 ± 0.50 cm and 0.73 ± 0.57 cm in diameter. Based on these findings, it can be concluded that the selected LasR inhibitors has an effective antiquorum sensing ability against *P. aeruginosa PAO1*.

Phase III - Gene expression analysis of quorum sensing regulatory genes in *Pseudomonas aeruginosa*

In this phase of study, the antagonistic efficiency of the selected compounds at transcriptional level was evaluated using an *in vitro* β-galactosidase reporter gene assay. For this purpose, *Escherichia coli* DH5α cells transformed with the plasmid pKDT17 was utilized as a reporter which linked the LasR gene to a *lasB::lacZ* translational fusion. The LasR expression was measured as the amount of β-galactosidase enzyme activity. Effect of the compounds C1, C2, and C3 on the LasR transcriptional activity was studied in presence of native autoinducer, acyl homoserine lactone (AHL).

The findings showed that in presence of AHL, the selected LasR inhibitors decreased the production of the β-galactosidase enzyme as the concentration increased. It was observed that there was a significant reduction in the β-galactosidase enzyme activity at a concentration of 100 nM. The β-galactosidase enzyme activity (expressed in Miller units) at 100 nM concentration for C1, C2, and C3 were found to be 54.46 ±

11.07, 29.18 ± 2.25, and 74.21 ± 0.23, respectively. The basal level activity of the β-galactosidase enzyme was found to be 7.8 ± 0.21 Miller units. These results suggested that the selected inhibitors could inhibit LasR by preventing it from binding to its target *lasB* promoter, which is necessary for LasR expression.

Quantitative real-time PCR (qRT-PCR) was done to validate the relative gene expression of the quorum sensing regulatory genes namely *lasI, lasR, rhlI, rhlR, pqsA,* and *pqsR*. The results showed that ½ sub-MIC of selected compounds, downregulated the regulatory genes of quorum sensing. Specifically, C1 significantly reduced the expression of quorum sensing regulatory genes when compared to C2 and C3.

Phase IV - Evaluation of selected LasR inhibitors on quorum sensing mediated virulence factors of *Pseudomonas aeruginosa*

The production of virulence factors such as pyocyanin, rhamnolipid, alkaline protease, lipase, protease, and elastase by *P. aeruginosa PAO1* were assessed in this phase of study because their production has been identified as an indicator of active quorum sensing. Pyocyanin is a toxic secondary metabolite that contributes significantly to *P. aeruginosa* infections through its effects such as reactive oxygen species (ROS) generation, redox sensitive lysosomal destabilisation, and DNA damage. The amount of pyocyanin produced was observed by measuring their absorbance at 520 nm. In this context, C1 inhibited pyocyanin production with the highest inhibition percentage of 78.51% at its ½ sub-MIC and 73.16% at its ¼ sub-MIC, compared to C2 whose inhibition at ½ and ¼ sub-MICs were found to be 77.53% and 67.93%. The ½ and ¼ sub-MICs of C3 exhibited an inhibition of pyocyanin as 68.85% and 65.40%, respectively.

The production of rhamnolipid by *P. aeruginosa* is required for adherence to host cells. The amount of rhamnolipid was determined by measuring their absorbance at 421 nm. The selected compounds were found to inhibit rhamnolipid production by up to 73.97%, 69.09%, and 71.27% at ½ sub-MICs of C1, C2, and C3, respectively. In case of ¼ sub-MICs of C1, C2, and C3, the amount of rhamnolipid produced was reduced with an inhibition percentage of 68.94%, 64.49%, and 67.23%, respectively. The alkaline protease and lipase production by *P. aeruginosa PAO1* as a result of quorum sensing was also altered on treatment with the selected compounds at its ½ and ¼ sub-MICs. The alkaline protease and lipase production was quantified by measuring their absorbance at 590 nm and 400 nm, respectively. C1, C2, and C3 at their ½ sub-MICs inhibited alkaline protease activity by 70.12%, 67.78%, and 68.11%, respectively whereas it was 62.09%, 54.14%, and 51.17% at their ¼ sub-MICs level which indicated a dose-dependent inhibition in their activity.

In terms of lipase activity, the production of lipase by *P. aeruginosa PAO1* was inhibited by 78.46%, 76.73%, and 72.63% at ½ sub-MICs of C1, C2, and C3, respectively. On treatment with ¼ sub-MICs of C1, C2, and C3, lipase activity was decreased by 76.89%, 75.72%, and 71.26%, respectively. The production of protease by *P. aeruginosa* improves colonisation of host tissues and plays a key role in biofilm development.

Thus, the effect of the selected compounds on protease production by *P. aeruginosa PAO1* was assessed by measuring the zone of clearance (cm) on skim milk agar plates. *P. aeruginosa PAO1* exhibited an inhibition in protease production after treatment with compounds with a diameter of zone of clearance of 0.51 ± 0.23 cm, 0.61 ± 0.23 cm, and 0.60 ± 0 cm for C1, C2, and C3, respectively at their ½ sub-MICs compared to untreated control (1.46 ± 0.47 cm). A similar trend was observed in the plates treated with ¼ sub-MICs of C1, C2, and C3.

Production of elastase is critical in inhibiting the host's inflammatory response during infections. Thus, the elastase activity of *P. aeruginosa PAO1* was quantified using qRT-PCR by measuring the relative gene expression of the target gene *lasB*, which is required for elastase production. The selected LasR inhibitors significantly reduced the elastase activity of *P. aeruginosa PAO1* by suppressing *lasB* expression. Based on the above results, it was evident that the selected LasR inhibitors strongly inhibited the production of virulence factors in *P. aeruginosa PAO1*.

Finally, the cytotoxicity of the selected LasR inhibitors were assessed using normal peripheral human blood lymphocyte cells, and cell viability was determined using MTT reduction assay. The findings indicated that none of the compounds were cytotoxic and had cytotoxicity of 28.6%, 29.04%, and 25.6% for C1, C2, and C3, respectively. Thus, these compounds C1, C2, and C3 could effectively disrupt the quorum sensing network of *P. aeruginosa PAO1* by inhibiting LasR without causing toxicity.

Conclusion

In conclusion, the study identified three potential inhibitors against LasR through a virtual screening approach for *P. aeruginosa* infections. *In vitro* validations have successfully demonstrated their antibiofilm and antiquorum sensing ability. The significant reduction in LasR gene expression at transcriptional level and downregulated gene expression pattern of quorum sensing regulatory genes further confirmed the antagonistic activity of selected inhibitors. The inhibitory activity of compounds on virulence factors production and their lower cytotoxic effects suggested that these compounds could be used as drug leads for designing effective quorum sensing inhibitors to treat *P. aeruginosa* associated infections.

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